ELECTRICAL STIMULATION AND RAPID CHILLING OF PIG CARCASSES

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INTRODUCTION

The primary objective of electrical stimulation (ES) of animal carcasses is to prevent cold shortening and consequent toughening in the meat. It is widely accepted for improving eating quality of beef and lamb, but has been less used with pig carcasses where the rate of post mortem muscle glycolysis is faster. Despite the earlier onset of rigor, cold toughening has been observed in pigmeat by several workers (Marsh et al., 1972; Fisher et al., 1980; Gigiel and James, 1984; Dransfield and Lockyer, 1985; Møller and Vestergaard, 1987; Barton et al., 1987). Consequently, over the last eight years, studies have been carried out on the effect of ES on guality characteristics of pig meat, in combination with accelerated or conventional chilling procedures.

In many cases, the results of these studies have been conflicting. Smith et al. (1980), Johnson et al. (1982), Swasdee et al. (1983), Crenweldge et al. (1984 a, b) and Neel et al. (1987) concluded that ES had no general effect on quality characteristics. On the other hand, Wiley (1986) found that loin chops from ES carcasses were less tender and juicy than those from non-stimulated (NES) controls, and that the cumulative weight losses were higher after ES. Reagan et al (1984, 1985) reported that ES carcasses which were chilled slowly at 7°C, gave more drip loss than NES controls. Gigiel and James (1984) reported no difference in drip loss in retail packs between rapidly and slowly chilled pig sides, but ES significantly increased drip fourfold in rapidly chilled, and more than fivefold in conventionally chilled sides. They also reported no significant difference in drip loss from muscle core samples from pigs chilled at different rates, but again ES increased the average drip loss from 1.6% to 4.6%.

The present study investigates the effect of ES on pH fall, drip loss and tenderness in the M. *l. dorsi* (LD) and M. *semimembranosus* (SM) from pig carcasses of different weights, chilled rapidly or conventionally.

MATERIAL AND METHODS.

Material

A total of 80 Large White pigs, ranging in weight from 38 kg to 82 kg were used in five groups of 16 according to the experimental plan shown in Table 1. All pigs were electrically stunned (400v for 4 sec) and slaughtered within 5h of delivery at the Institute abattoir.

Table 1 Schedule of chilling treatments for 5 experimental groups of pigs with electrical stimulation (ES) and without (NES). Comparisons are made between sides of each pig.

	Wt. range Kg	Mean Wt. Kg	Comparison
1	38-57	46	
2	39-58	47	ES v NES, rapid
3	64-71	67	chill
4	64-80	71	ES rapid v NES
5	62-82	75	conventional chill

PROCEDURES

Electrical stimulation

Preliminary trials with 60 pigs using low voltage ES immediately after slaughter and high voltage (12.5Hz and 25Hz) at 20 min. post-slaughter, showed that the greatest and most consistent pH fall was produced by 700v peak at 12.5Hz, applied for 90 sec. at 20 min post-slaughter. These parameters were used in this study, and ES was applied to half-carcasses via stainless steel hook electrodes inserted above the Achilles tendon and in the severed neck muscles.

Chilling

All pig sides, ES and NES, were placed in chillers at 40 min post-slaughter. For rapid chilling, sides were held in a room with air at -9° ± 3°C and 1.2 ± 0.5 m/sec velocity, until temperature in the deep loin reached 7° - 10°C. This took between 1.6h and 2.0h depending on carcass weight, and sides were then transferred to a conventional chiller with air at 2°C and 0.5 m/sec velocity. Conventionally chilled sides were held in the aforementioned chiller for the whole duration of cooling. During chilling, temperatures were recorded in the deep and surface musculature of the loins and legs. All pig sides were butchered at 48h post-slaughter to provide muscle samples for assessment.

Table 2. Mean pH values at different times post slaughter for LD and SM for stimulated (ES) and non-stimulated (NES) pig sides. Eacn value is the mean for 16 sides.

(a) LD

	20 min*		40 min		3h		24h	
	NES	ES	NES	ES	NES	ES	NES	ES
1	6.32	6.34	6.13	6.13	5.97	5.83	5.50	5.48
2	6.49	6.50	6.36	6.17	6.10	5.81	5.58	5.55
3	6.59	6.55	6.53	6.44	6.24	5.94	5.60	5.50
4	6.31	6.24	6.15	6.02	5.79	5.69	5.47	5.42
5	6.43	6.46	6.28	6.10	6.00	5.82	5.61	5.61
overall mean	6.44	6.42	6.29	6.17	6.02	5.82	5.55	5.51

(b) SM

1	6.60	6.62	6.46	6.29	6.25	5.88	5.51	5.55
2	6.64	6.64	6.53	6.14	6.36	5.95	5.66	5.55
3	6.75	6.67	6.72	6.47	6.47	6.10	5.59	5.62
4	6.64	6.49	6.47	6.15	6.22	5.84	5.57	5.47
5	6.65	6.66	6.50	6.13	6.30	5.83	5.67	5.65
overall mean	6.66	6.62	6.54	6.24	6.32	5.92	5.60	5.68

* Before stimulation

Table 3. Mean values of drip loss (48H), shear value and FOP on LD and SM muscles. Each value is the mean for 16 sides

	Drip loss (% by wt)				Shear value (Kg)				FOP	
	LD		SM		LD		SM		LD	
	NES	ES	NES	ES	NES	ES	NES	ES	NES	ES
1	3.06	1.98	1.93	2.01	8.25	7.46	7.17	6.04	23	20
2	2.34	1.31	1.14	1.30	9.01	6.22	7.17	6.29	18	19
3	2.37	1.99	1.49	1.90	5.95	5.17	5.75	5.61	24	25
4	3.25	2.28	1.91	1.95	6.37	5.60	5.66	5.65	32	33
5	2.20	1.35	1.20	1.50	7.74	6.38	5.50	5.58	29	26
overall mean	2.64	1.78	1.53	1.73	7.46	6.17	6.25	5.83	25	25

Table 6. Relationship between drip loss and ΔpH_{40} of LD. Drip losses(%) are grouped in 3 ranges.

Drip %	n	Δph_{40} (mean)	SD
0.60-1.2	26	0.18	0.11
1.21-2.2	29	0.15	0.13
2.21-5.5	23	0.08	0.10

In Table 7, samples are grouped together into 4 ranges according to shear values. The ranges may be considered as 'very tender' (2.2 - 4.5), 'tender' (4.51 - 6.3), 'tough' (6.31 - 8.2) and 'very tough' (8.21 - 16.0). The distribution of ES and NES samples among the ranges demonstrates the general improvement resulting from ES, although individual samples varied widely. Again, the lower drip loss from ES samples is clearly shown.

Table 7. Relationship between shear value, pH_{40} and drip (48h) of LD muscles. Shear values are grouped in ranges regardless of ES.

Shear range(Kg)		n	pH_{40} (mean)	% drip (mean)
2.20-4.5	NES	6	6.60	1.76
	ES	20	6.21	1.43
4.51-6.3	NES	11	6.41	2.41
	ES	30	6.21	1.61
6.31-8.2	NES	35	6.30	2.73
	ES	15	6.08	2.15
8.21-16.0	NES	25	6.24	2.71
	ES	15	6.12	2.14

DISCUSSION

Most previous studies on the effect of ES on quality characteristics of rapidly and slowly chilled pig carcasses have produced conflicting results. In some cases this may be because of variation between animals, but it could also have resulted from the different ES and chilling parameters employed. This study, in which treatment were compared between sides of the same animal and used a total of 80 pigs, directly contradicts some of the main conclusions of previous workers.

The effect of ES on pH at 40 min post-slaughter varied widely between animals. Table 4 shows that, for about 27% of the pigs, ES produced little or no pH drop; for another 27% the drop was slight, and in fact only about 12% of the pigs gave drops >0.25 pH units. the effect of high voltage ES on pH was therefore less than that found by Gigiel and James (1984) where the mean pH of stimulated LD at 40 min post-slaughter was less than 5.60. In their study, however, high voltage (700v peak) was applied at 5 min post-slaughter and this early stimulation may have contributed to the greater pH fall compared to our study where ES was applied 20 min after slaughter.

The most interesting result and possibly the most contradictory, was the effect which ES had on drip loss. Most workers have reported increased drip loss from ES meat, but in our experiments, drip was consistently and significantly lower from ES LD samples. Over all pigs, drip loss from the LD of NES sides ranged from 2.2 to 3.3% compared with 1.3 to 2.3% from their ES counterparts. Table 4 shows quite clearly that, even where ES had little apparent effect on pH, drip was consistently lower from sides which had been stimulated. Where pH_{40} values for ES and NES sides were the same, the amount of drip was still significantly less after ES. This effect was not observed with SM samples, where ES induced a greater pH fall than in the LD, and led to a slight increase in drip loss.

Examination of Table 6 shows that although approximately 70% of samples had similar ΔpH_{40} values, drip ranged from 0.6 to 2.2%. For the remaining 30%, minimum ΔpH_{40} was accompanied by maximum drip loss. Gigiel and James (1984) reported a fourfold increase in drip from ES LD compared with NES controls, although their drip measurement was over 24h instead of 48h period of this study. As stated earlier, the pH drop observed in their experiments was also decidedly greater.

It is quite clear that ES improved tenderness in the LD, and to a lesser extent in the SM. Although the variation between pigs was considerable, the effect of ES on the LD was particualrly consistent across all 5 groups of pigs. The mean overall improvement in tenderness of the LD was 17%, which could be commercially important for high value loin cuts. For the SM, the improvement was only 6%. The greatest improvement observed (30%) was in the 5th group of pigs where ES, rapidly chilled sides were compared with NES, conventionally

pH

pH was measured in 1g samples of LD and SM removed from the carcasses at 20 min, 40 min, 3h and 24h, and homogenised with 10ml iodoacetate solution.

Fibre optic probe

The light scattering proportions of LD samples from all carcasses were measured at 24h post-slaughter using a fibre optic probe (FOP) (MacDougall, 1984).

Drip

Sections of LD (2.5 x 5 x 5cm) and SM were assessed for drip loss at 48h post-slaughter by suspending them in plastic mesh inside polythene bags held at 1°C. The weight loss from the muscle samples was recorded over a further 48h period.

Tenderness

At 3 days post-slaughter, blocks of LD and SM measuring (5 x 5 x 5cm) were vacuum packed, cooked in a water bath at 80°C to a centre temperature of 72°C, and cooled overnight in cold running water. From each cooked muscle, 6 blocks (2 x 1 x 1cm) were cut longitudinally in fibre direction and sheared by Volodkevitch jaws on a Stevens Compression Tester. Peak shear force was recorded to indicate relative toughness.

RESULTS

Rapid chilling reduced temperature in the deep leg to 10°C within 5.8h to 10.8h post-slaughter, and in the deep loin to 10°C within 2.1 to 3.0h, depending on carcass size. Conventional chilling cooled the deep leg to 10°C in 11h and the deep loin in under 5h. Table 2 shows the changes in pH in the LD and SM during cooling for each of the 5 experimental groups. The values at 20 min, measured before stimulation, show the general low level of pH in the pigs at this stage. The comparatively slight effect of ES can be seen in the mean pH values 6.17 (ES) and 6.29 (NES) at 40 min with SD ranging from 0.12 to 0.30 and 3h for the LD. The effect was slightly greater in the SM with 6.24 (ES) and 6.54 (NES) with SD ranging from 0.14 to 0.29). Overall, the ES-induced pH changes for the LD at 40 min and 3h were 0.12 and 0.30 respectively, and for the SM were 0.20 and 0.40 respectively.

Table 3 lists mean data for drip loss within the 5 experimental groups and shows that, for the LD, the ES sides consistently had less drip than NES sides. The SM comparison shows a slight increase in drip with ES. The relative tenderness of LD and SM samples are also listed in Table 3, and it can be seen that, in each group, the ES LD had lower shear values than NES. The corresponding improvement in tenderness was only slight in the SM samples. The FOP values were typical of normal pork muscle, with no indication of either PSE or DFD conditions.

Table 4. Influence of ΔpH_{40} (NES-ES) on ΔDrip (NES-ES) of LD muscle. Samples grouped according to pH_{40} values.

ΔpH_{40}	n	ΔDrip (mean)	SD
0.00-0.05	22	0.99	0.77
0.05-0.15	23	1.04	0.91
0.15-0.25	23	0.77	0.70
0.25-0.45	10	0.88	0.74

The influence of the induced pH change at 40 min post-slaughter (ΔpH_{40}) on the difference in drip from ES and NES muscles (ΔDrip) from pigs in each ES/chilling treatment is seen in Table 4. For about 27% of the pigs there was little or no difference in pH with ES; only for about 12% of pigs was ΔpH_{40} more than 0.25 units. This table shows clearly that drip from ES sides was significantly less than from NES sides. Over all treatments, the mean drip loss from the LD of ES sides was 1.78% compared with 2.64% from NES sides. For the SM, the corresponding values were 1.73% and 1.53%.

Table 5. Influence of pH_{40} on the amount of drip (48h) from LD muscle from NES and ES pigs.

pH_{40}		n	Drip (mean)	SD
5.50-6.10	NES	18	3.10	1.18
	ES	27	2.02	1.05
6.11-6.30	NES	13	3.05	1.00
	ES	33	1.55	0.84
6.31-6.86	NES	46	2.31	0.88
	ES	19	1.76	0.67

In Table 5 the mean drip values are grouped according to 3 ranges of pH_{40}, regardless of ES. It can be seen that, where NES and ES samples are within same pH_{40} range, the drip is consistently lower from the ES samples.

chilled sides. This demonstrates the potential advantage of an ES, rapid chill treatment over conventional chilling.

The improvement in tenderness contradicts the results of Wiley (1986) who found less tender pork chops after ES and the workers who found no effect from ES. Our results do, however, agree in this respect with those of Gigiel and James (1984) who attributed the tenderising effect to possible avoidance of a degree of cold shortening from rapid chilling.

Grouping samples into shear value ranges (Table 7) shows that the two "tender" ranges contain 50 ES samples and only 17 NES. By contrast, the two "tough" ranges contain 30 ES samples and 50 NES. There was no significant difference in LD shear values between conventionally chilled sides and rapidly chilled sides, even though the rapid chilling reduced the LD to a temperature where cold-shortening was a possibility.

This study has demonstrated that high voltage ES, applied 20 min after slaughter, improved tenderness of the LD and, to a lesser extent, the SM of rapidly chilled pig carcasses. This advantage was obtained without evidence of PSE characteristics in the form of pale muscle or increased drip loss. In fact, in this study, ES had the added advantage of consistently reducing drip loss in the loin muscle.

REFERENCES

Barton-Gade, P., Bejerholm, C. and Borup, V. (1987):
Influence of different chilling procedures on the eating quality of pork chops. Proc. 33rd Int. Cong. Food Sci. and Technology, 181, Helsinki.

Bendall, J.R. and Fentz, V. (1978):
Investigation of the effect of ES on biochemical changes and the formation of drip in the muscles of pig carcasses. Visiting Workers' Report No 8, Meat Research Institute, Langford, Bristol.

Crenwelge, D.D., Terrell, R.N., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. (1984):
Effects of time post-mortem of ES and post-mortem chilling method on pork quality and palatability traits. J. Food Sci. 49, 294.

Crenwelge, D.D., Terrell, R.N., Dutson, T.R., Smith, G.C. and Carpenter, Z.L. (1984):
Effect of chilling method and ES on pork quality. J. Anim. Sci. 50,

Dransfield, E. and Lockyer, D.K. (1985):
Cold-shortening toughness in excised pork M. *longissimus dorsi*. Meat Science, 13, 19.

Fischer, C., Honikel, K.O. and Hamm, R. (1980):
Cold shortening in the dark and light musculature of cattle and pigs. Fleischwirtschaft, 60 (2), 263.

Gigiel, A.J. and James, S.J. (1984):
ES and ultra-rapid chilling of pork. Meat Science, 11, 1.

Gigiel, A.J. Butler, F. and Hudson, W.E.:
Alternative methods of pig chilling. Meat Science (in press).

Honikel, K.O. and Reagan, J.O. (1985):
Hot boning of pig carcasses : influence of chilling conditions on meat quality. In: Accelerated Processing of Meat (edit. A.Romita, C.Valin and A.A. Taylor) p.97. Elsevier Applied Science. London

Honikel, K.O. and Reagan, J.O. (1986):
Influence of different chilling conditions on hot-boned pork. J. Food Sci. 51, 766-768.

Honikel, K.O. (1986):
Influence of chilling on biochemical changes and quality of pork. Proc. IIR. Symposium 'Meat Chilling', Bristol.

James, S.J. and Gigiel, A.J. and Hudson, W.R. (1983):
The ultra-rapid chilling of pork. Meat Science, 9, 63-78.

Johnson, D.D., Savell, J.W., Weatherspoon, L. and Smith, G.C. (1982):
Quality, palatability and weight loss of pork as affected by ES. Meat Science. 7, 43-50.

Macdougall, D.B. (1984):
Meat Research Institute Light Probe for Stressed Meat Detection, Anal. Proc. 21, 494-5.

Marsh, B.B., Cassens, R.G., Kaufman, R.G. and Briskey, E.J. (1972):
A research note Hot Boning and Pork Tenderness. J. Food Sci. 37, 179.

Miller, K.A., Reagan, J.O., Cordray, J.C., Abu-Bakar, A., Huffman, D.L. and Jones, W.R. (1987):
Comparison of hot processing systems for pork. J. Anim. Sci. 58,

Møller, A.J. and Vestergaard, T (1988):
Effect of temperature conditioning on toughness in hot boned pork loins with high or low initial pH. Proc. 34th Int. Cong. Meat Sci. and Technology, Brisbane.

Neel, S.W., Reagan, J.O. and Mabry, J.W. (1987):
Effects of rapid chilling and accelerated processing on the physical and sensory characteristics of fresh pork loins. J. Anim. Sci. 64, 765-773.

Reagan, J.O. (1983):
Optimal processing systems for hot-boned pork. Food Technology. 5, 79.

Reagan, J.O., Carpenter, J.A. and Honikel, K.O. (1984):
Rapid processing systems for pork. Proc. 30th Europ. Meeting Meat Res. Workers, Bristol.

Reagan, J.O. and Honikel K.O.(1985):
Weight loss and sensory attributes of temperature conditioned and electrically stimulated hot processed pork. J. Food Sci. 50, 1568.

Smith, G.C., Savell, J.W., Dutson, T.R., Hostetler, R.L., Terrell, R.N., Murphey, R.N. and Carpenter, Z.L. (1980):
Effects of ES on beef, pork, lamb and goat meat. Proc. 26th Europ. Meeting Meat Res. Workers, Colorado Springs, H-5, 19-22.

Stoler, S. (1986):
Warm boning of pigs in relation to meat tenderness. Proc. 32nd Europ. Meeting Meat Res. Workers, Ghent, 89-90.

Swasdee, R.L., Terrell, R.N., Dutson, T.R., Crenwelge, D.D. and Smith G.C. (1983):
Processing properties of pork as affected by ES, post-slaughter chilling and muscle group. J. Food Sci. 48, 150.

Taylor, A.A. and Dant, S.J. (1971):
Influence of carcass cooling rate and drip loss in pig meat. J. Fd. Technol. 6,(2),131.

Wiley, E. (1986):
The effects of chilling and ES methods on hot-processed pork and the utilisation of ES and non-ES hot processed pork in cured meat products. Dissertation Abstracts International, B, 46(7) 2174, order no. DA8519683.

THE EFFECT OF HOT VS COLD BONING ON THE QUALITY OF RETAIL CUTS PREPARED FROM PRIMALS FROM SKINNED PIG CARCASSES

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SUMMARY
The effects of hot boning on the microbiological and sensory quality of skinned pork primals were evaluated. Hot boned primals, chilled for one day, and cold boned primals were cut up into retail cuts which were vacuum packaged and stored at 2±2°C.
Hot boning resulted in markedly less total carcass weight loss, 0.9% higher meat yields and a similar sensory quality as compared with cold boning. Hot boning primals had significantly higher colony counts. However, this did not affect the bacteriological quality of retail cuts. It is concluded that hot boning might be a good alternative for cold boning provided that, by strictly adhering to Good Manufacturing Practices, one achieves levels of microbial decontamination that are well below the levels generally considered to be safe.

INTRODUCTION

Hot boning of pork has traditionally been practiced in (Eastern) Europe in the processing of meat products. However, hot boning may also be beneficial for the production of fresh pork (Reagan, 1983; Smulders & Van Laack, 1988). Major advantages claimed by the scientific literature are less refrigeration costs (Henrickson, 1982; Cutbertson, 1980), higher turnover (Henrickson, 1982), better water-binding resulting in lesser drip formation (Honikel & Reagan, 1987; Woltersdorf & Troeger, 1987). Amongst others, effects of hot boning on meat quality are attributed to a faster chilling rate of hot boned primals as compared with the meat on the carcass (Daudin & Culioli, 1987; James, 1987). Modern pig slaughter technology relies on the scalding and singeing of the animal. The thermal stress, that results from these dehairing procedures may have a negative influence on meat quality (Takacs & Biro, 1988). Furthermore, scalding water may increase the microbial contamination of the carcass and thus result in a shorter storage life of the pig meat (Schaeffer-Seidler et al., 1984; Jones et al., 1984).
It has been suggested (Takacs & Biro, 1985; Troeger & Woltersdorf, 1987) that skinning of pig carcasses might contribute to the production of meat with a very low bacterial load, Also, Troeger and Woltersdorf (1987) reported that meat from skinned pig carcasses had a better sensory quality than meat from carcasses that had been scalded.

The purpose of this study was to evaluate if the meat quality of skinned pig carcasses from the Dutch commercial supply, might be further improved by hot deboning. In addition, the bacteriological condition of the hot boned primal- and retail cuts was monitored.

MATERIALS AND METHODS

In a pilot plant 8 large White/Dutch Landrace cross-bred pigs were slaughtered and skinned with a vertical drum skinner. All righthandside primals were excised within 1 h post mortem. Cold boning of the left handside primals was conducted after overnight storage at 1±1°C. After every two carcass sides cutting tables were cleaned, disinfected and dried. Hot boned primals were wrapped in a O_2-permeable film for one day to avoid rapid desiccation. Cold boned primals (immediately after deboning) and hot boned primals (after 1 day of chilling) were cut up into retail cuts which were vacuum packaged in a film with low O_2-permeability (<30 ml $O_2/m^2/24$ h at 1 atm at 25°C). After 7 days of storage at 2±2°C the meat was unpacked and the sensory quality traits assessed according to the procedures described by Smulders (1986).
The shoulder (M. triceps brachii) and belly were sampled for purposes of bacteriological monitoring, relying on the method described by Van Laack and Smulders (1988). At day 0 and 1 pri-

mals were sampled on the outer surface. At day 7 retail cuts were sampled on the cut surface.
Unless indicated otherwise, comparisons between hot and cold boning were made between muscles within a carcass. Statistical significance of differences was tested by Student t-test ($p<0.05$; pair-wise were appropiate).

RESULTS AND DISCUSSION

Table 1 Carcass yield of hot boned righthandsides and cold boned lefthandsides of skinned pig carcasses as assessed by weighing immediately before and after boning (expressed as %)

	Hot boned	Cold boned
Meat yield	63.5[a*]	62.3[b]
Fat yield	14.6	14.3
Bone yield	10.8	10.4
Total weight loss	0.25[a]	1.81[b]

* means with different superscripts differ significantly ($p<0.05$).

In Table 1 the yields of hot vs cold boned carcass sides are presented. The total weight loss after hot boning was significantly lower than after cold boning ($p<0.05$). We attribute this difference mainly to the increased meat yield which was 1.2% higher after hot than after cold boning.
It was very difficult to prepare retail cuts from hot boned primals when these were still warm. The resulting cut distortion was unacceptable. Therefore the hot boned meat was chilled for one additional day before cutting was started. This extended chilling period was expected to reduce the economic benefits of hot boning because of moisture loss through evaporation and drip. Yet, as can be seen from Table 2, the maximal drip-loss during the day storage was only 0.25%. Hence, the total difference in meat yield was 0.9% in favour of hot boning.
We anticipated that hot boning would lead to faster chilling rates,and therefore to minimal rates of protein denaturation (Penny, 1977; Tarrant, 1977; Taylor et al., 1980-1981). The latter would lead to an increased waterholding capacity and thus to less drip formation. However, in the present experiment the differences between hot and cold boning were very small

Table 2 Drip losses of hot boned primals during 1 day of storage at 1±1°C (n=8 except where indicated) (%)

Ham	0.16±0.06
Shoulder	0.22±0.09 (n=7)
Loin	0.22±0.05
Tenderloin	0.25±0.13 (n=7)
Belly	0.17±0.03

Table 3 Drip losses of retail cuts from hot and cold boned primals vacuum packaged after 7 days of storage (%)

	Hot boned	Cold boned
Ham (M. semimembranosus)	5.9	6.1
Loin (M. longissimus)	3.7	4.4
Shoulder (M. triceps brachii)	3.7[b]	2.6[a*]
Belly	2.2	1.7

* means with different superscripts differ significantly ($p<0.05$).

and loins lost less weight than cold boned counterparts; cuts from shoulders and bellies on the other hand lost more weight after hot than after cold boning. The absence of significant differences is probably explained by the excellent quality of the cold boned meat. At 80 min post mortem the loin pH was 6.55 at a muscle temperature of 30°C. Thus the waterholding potential of the cold boned meat was very high to start with so that hot boning could add little more. Although pH-fall was relatively slow, skinning and hot boning did not induce cold shortening. Sarcomere lengths of hot and cold boned loin samples were similar (Table 4). Shear forces of hot

boned loins were slightly, but insignificantly, lower than those of the cold boned ones (Table 4).

Table 4 Sarcomere length and shear force of hot and cold boned longissimus dorsi cuts after 7 days of vacuum storage at 1±1°C (n=8)

	Hot boned	Cold boned
Shear force ($kg\ cm^{-2}$)	4.20	4.65
Sarcomere length (μm)	1.75	1.74

From the point of view of yield and sensory meat quality, hot boning of skinned pig carcasses seems to be feasible. Before such novel slaughter and processing techniques are introduced widely, it is imperative to establish if hygienic drawbacks might ensue.
Tables 5a and 5b include the results of the bacteriological examination of the hot and cold boned meat. Hot boned primals had significantly higher colony counts than cold boned primals. This may be due to the sticky surface and the higher temperature of the hot meat which could have led to higher initial levels of contamination (Smulders & Eikelenboom, 1987). Furthermore there was a considerable increase (>0.5 log/cm^2) in colony counts during

Table 5a Microbiological condition (log/cm^2) of hot and cold boned primals [belly (B) and shoulder (S) (day 1)]

		Primals	
		Hot boned	Cold boned
Aerobic mesophilic colony count	B	3.92^{b*}	3.13^{a}
	S	3.44^{b}	2.56^{a}
Enterobacteriaceae	B	2.45^{b}	1.55^{a}
	S	2.36^{b}	1.86^{a}
Lactic acid bacteria	B	3.22^{b}	2.58^{a}
	S	3.18^{b}	2.48^{a}

* means with different superscripts differ significantly ($p<0.05$).

Table 5b Microbiological condition (log/cm^2) of hot and cold boned retail cuts [belly (B) and shoulder (S) (day 7)]

		Retail cuts	
		Hot boned	Cold boned
Aerobic mesophilic colony count	B	3.20	2.99
	S	2.35	2.14
Enterobacteriaceae	B	2.18	2.17
	S	1.63	1.50
Lactic acid bacteria	B	2.58	3008
	S	3.09	2.93

* means with different superscripts differ significantly ($p<0.05$).

one day of storage. Probably the circumstances for microbial growth would have been smaller, had the hot boned meat been vacuum packaged immediately after excision (Apple & Terlizzi, 1983). Clearly, vacuum packaging with the purpose of storage for only one day is far too expensive in meat industry practice and would reduce the economic benefits of hot boning considerably.
The contamination of both hot and cold boned meat was well below the levels generally considered acceptable for conventionally produced pork (Salm et al., 1978). The experimental procedure followed does not allow for deciding whether these levels were the result of skinning or of the intensified cleaning and disinfection procedure.
Colony counts on retail cuts from hot and cold boned primals were similar. Differences existing on primals did not affect the quality of the retail cuts significantly. This is in agreement with the findings of Greer et al. (1983) who showed that hygiene during retail cutting was far more important than the initial contamination of primals to be cut.

CONCLUSION

Hot boning of skinned pig carcass followed by retail cutting after one day of refrigerated storage, results in markedly less weight loss and similar sensory meat quality as cold boning. Microbiological monitoring indicates

that hot boned meat might represent a greater risk. By strict adherence to Good Manufacturing Practices one might still achieve contamination levels that are well below the levels generally considered to be safe.

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REFERENCES

Apple, J.M. & Terlizzi, F.M. (1983):
Packaging systems for hot-boned beef. Food Technol. 37, 68-77.

Cuthbertson, A. (1980):
Hot processing of meat: a review of the rationale and economic implications. Pp. 61-68. In: R. Lawrie (Ed.). Developments in meat science - 1. Appl. Sci. Publ., London.

Daudin, J.D. & Culioli, J. (1987):
Chilling of hot boned muscles: cooling and weight loss kinetics. In: A. Romita, C. Valin, and A.A. Taylor (Eds). Accelerated processing of meat. Elsevier Appl. Sci. Publ., London, pp. 29-48.

Greer, G.G., Jeremiah, L.E. & Weiss, G.N. (1983):
Effects of wholesale and retail contamination on the case life of beef. J. Food Prot. 46, 842-845.

Henrickson, R.L. (1982):
Hot processing in the United States. Pp. 169-180. In: K.R. Franklin and H.R. Cross (Eds). Meat Science and Technology International Symposium Proceedings. Natl Livestock and Meat Board, Chicago (Il).

Honikel, K.O. & Reagan, J.O. (1987).
Hot boning of pig carcasses: influence of chilling conditions on meat quality. In: A. Romita, C. Valin and A.A. Taylor (Eds). Accelerated processing of meat. Elsevier Appl. Sci. Publ., London, pp. 97-100.

James, S.J. (1987):
Efficient systems for rapid cooling of carcasses and hot-boned meat. Pp. 49-56. In: A. Romita, C. Valin and A.A. Taylor (Eds). Accelerated processing of meat. Elsevier Appl. Sci. Publ., London.

Jones, B., Nilsson, T. & Sorquist, S. (1984):
Kontamination von Schweineschlachtkörpern durch Brühwasser. Fleischwirtsch. 64, 1243-1246.

Laack, R.L.J.M. van & Smulders, F.J.M. (1988):
Effects of high temperature conditioning and air trapping on the keepability of hot and cold boned, vacuum-packaged, beef longissimus. Proc. 34th Internatl Congr. Meat Sci. Technol., Brisbane, Australia, pp. 539-541.

Penny, J.F. (1977):
The effect of temperature on the drip, denaturation and extracellular space of pork longissimus dorsi muscle. J. Sci. Food Agric. 28, 329-338.

Salm, C.P., Ray, F.K., Aberle, E.D., Okos, M.R. & Judge, H.D. (1978):
Yield charateristics, microbial load and chilling rates of skinned pork carcasses. J. Food Sci. 43, 1144-1152.

Schaefer-Seidler, C.E., Judge, M.D., Cousin, M.A. & Aberle, E.D. (1984):
Microbiological contamination of primal cut yields of skinned and scalded pork carcasses. J. Food Sci. 49, 356-358.

Smulders, F.J.M. (1986):
Sensory meat quality and its assessment. Vet. Quart. 8, 158-167.

Smulders, F.J.M. & Eikelenboom, G. (1987):
Accelerated meat processing; microbiological aspect. In: A. Romita, C. Valin and A.A. Taylor (Eds). Accelerated processing of meat. Elsevier Appl. Sci. Publ., London, pp. 79-93.

Smulders, F.J.M. & Laack, H.L.J.M. van (1988):
Carcass yield, meat quality and functional properties of pork as affected by hot processing procedures. Proc. 34th Internatl Congr. Meat Sci. Tech-

nol., Brisbane, Australia, pp. 613-615.

Takacs, J. & Biro, G.Y. (1985):
The effects of scalding and singeing on the quality of pig meat. World Assoc. Vet. Food Hygienist, 9. Internatl Symp., Budapest, Proc. Vol. 1, 180-185.

Tarrant, P.V. (1977):
The effect of hot boning on glycolysis in beef muscle. J. Sci. Food Agric. 28, 927-930.

Taylor, A.A., Shaw, B.G. & MacDougall, D.B. (1980-1981):
Hot deboning beef with and without electrical stimulation. Meat Sci. 5, 109-113.

Troeger, K. & Woltersdorf, W. (1987):
Influence of scalding and dehairing during pig slaughtering on meat quality. Fleischwirtsch. 67, 66-70.

Woltersdorf, W. & Troeger, K. (1987):
Hot boning and hot meat production from pig carcasses. I. Influence of hot boning on meat quality. Fleischwirtsch. 67, 574-576.

COLOUR AND COLOUR STABILITY OF HAMBURGERS PREPARED FROM ELECTRICALLY STIMULATED, HOT VS COLD BONED, CLOSELY TRIMMED BEEF

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INTRODUCTION

Several meat quality characteristics observable in the processing plant and supermarket, or measurable in the laboratory are known to be closely related to the consumers' appreciation of meats. Among these the colour of meat is of primary importance for the retailer as it largely determines the consumers' inclination to buy one product or brand rather than the other (Hood and Riordan, 1973). The colour of meat depends on a number of independent variables important in marketing, e.g., animal age, pre- and post-slaughter handling and the display environment (McDougall, 1977). All of these interfere heavily with the pure physics of colour observation by individuals or colour-analysing instruments. Therefore, novel ways of processing carcasses into meats need to be evaluated carefully as to their impact on meat colour.

The colour of meat primarily depends on the concentration of the pigment myoglobin which can exist in three forms: the purple (reduced) myoglobin (Mb), the cherry-red (oxygenated) oxymyoglobin (MbO) and the greyish-brown (oxidized) metmyoglobin (MMb). These three forms are constantly being interconverted. When the surface of meat is exposed to air, oxygen will penetrate into the interior, the oxygen diffusion being deeper the longer the meat is exposed. Below the layer of MbO, a thin Mb layer exists where the partial pressure of oxygen is too low for oxygenation to occur. Beyond that a thin layer of Mb is oxidized to MMb, which will gradually shift from interior to surface, thus causing the so-called 'fading' of meat (Seideman et al., 1984). Several factors determine the accumulation of MMb on the meat surface and hence the stability of meat colour, e.g. rates of oxygen-diffusion (Brooks, 1938) and -consumption (Atkinson and Follett, 1973), (auto) oxidation (Lawrie, 1979) and the enzymatic reduction of MMb (Hood, 1980; Ledward, 1985; Renerre and Labas, 1987). Extrinsic factors such as pH decline and temperature, water-holding capacity and muscle structure affect how colour is perceived (McDougall, 1977).

Several meat processing procedures markedly influence both intrinsic and extrinsic colour determinants. Two major ones are meat comminution and salting, the former one destroying the reducing system and thus promoting the rapid formation of MMb (Ledward et al., 1977), the latter one exerting a strong pro-oxidant effect (Huffman, 1980).

Many of the afore-mentioned factors that determine colour stability are affected by accelerated processing of meats. Firstly, electrical stimulation will induce a rapid early post-mortem pH decline, secondly hot boning accelerates the chilling rate, and finally pre-rigor comminution and salting largely determine the chemical form of Mb, rate of ATP breakdown and glycolysis and thus the water-holding capacity and light reflectance characteristics.

In earlier studies we investigated the influence of electrical stimulation and hot boning on the colour (stability) of intact bovine longissimus and psoas major muscle (Van Laack et al., 1989; Van Laack and Smulders, 1989a) and ground beef of a relatively high fat content (Van Laack and Smulders, 1989b). The purpose of the present study was to assess the impact of accelerated processing on the colour stability of a comminuted, pre-salted product prepared from beef from which all visible fat had been trimmed off.

MATERIALS AND METHODS

Eight Friesian Holstein cows, 3-5 years old, were stimulated electrically (85 V, 14 Hz, 30 s) within 5 min post mortem. At approximately 1 h post mortem the sternomandibularis muscles of randomly selected carcass sides were hot boned, cut up in

chunks, ground through a 3 mm plate, mixed with a hamburger-mix (Verstegen, Rotterdam, The Netherlands) resulting in a salt concentration of 1.8%, and blended in a Hobart blendor for 3 min. Using a mould, hamburgers (1.5 cm thick, 8 cm diameter) were hand-pressed and frozen at -40°C and finally stored at -20°C in card-board boxes. An identical procedure was followed for cold-processing of hamburgers, i.e. after the remaining carcass side had been chilled at -1 to -4°C, air velocity 3 m/s, during the first 90 min, whereafter the carcass was stored at 1±1°C, air velocity 0.5 m/s for 24 h.

Before freezing hamburgers were sampled for microstructural study using the methodology described by Koolmees et al. (1989).

Sixteen hamburgers per treatment group (2 samples per carcass-side) were thawed and allowed to bloom at 2±2°C for 2 days whereafter they were displayed for 7 days at 1±1°C under continuous illumination with a 300-400 Lux lamp (Philips TLC95). At 0, 2, 4 and 7 days of display L*, a*, b* values and spectrum (400-700 nm) were analysed instrumentally by means of a Hunter Labscan SN12244; 10° Standard Observer, D65 illuninant, 50 mm opening, calibrated with black and white tiles. Significance of differences were tested with the Student-t-test (paired where appropriate).

RESULTS AND DISCUSSION

Figure 1 includes the Hunter CIE-Lab values of hot and cold processed hamburgers during one week of display at 1±1°C.

Throughout the storage period L*, a* and b* values were higher in hot than in cold processed beef burgers, which differences were significant ($p<.05$) in all instances but two (a* values at day 0 and 2). The higher values for Hunter L*, denoting a brighter colour, are in agreement with earlier findings on pre-rigor ground (Van Laack and Smulders, 1989b) and flaked beef (Van Roon et al., unpublished results cited by Smulders et al., 1987).

Hot boned, intact beef and pork muscles almost invariably exhibit a darker colour than cold boned muscle

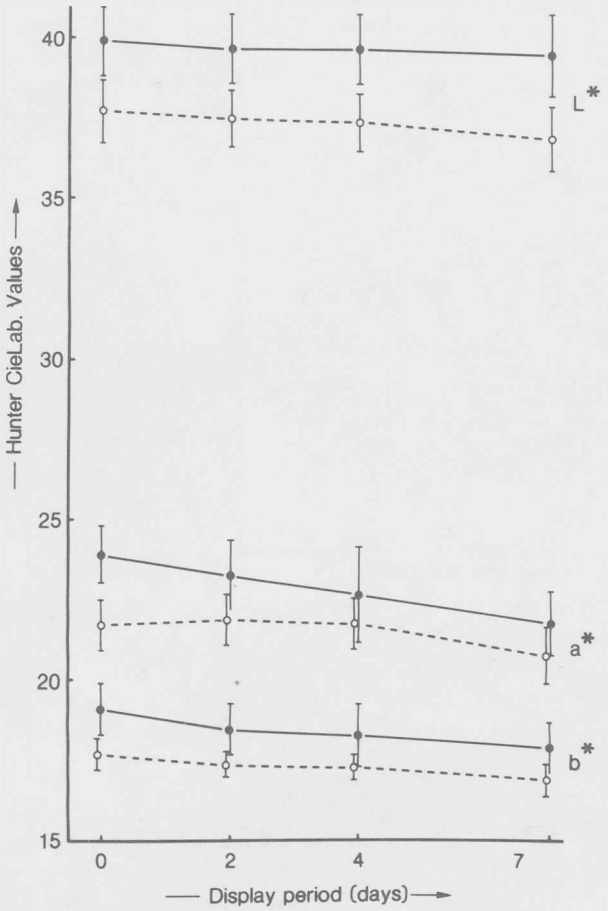


Fig. 1 Hunter CIE-Lab values of hot- (•) vs cold- (o) processed hamburgers during 1 week of display at 1±1°C

after one week of refrigerated vacuum storage (Smulders et al., 1988). This has been attributed to the superior waterholding properties of pre-rigor excised meat which might reduce light reflectance and, to a lesser degree, to protein denaturation caused by faster chilling (Taylor et al., 1980-1981). That the situation is reversed in comminuted beef with a relatively high percentage of fat was suggested to be the result of pre-rigor ground beef having soft warm fat which is dispersed through the product as small droplets; in cold processed beef burgers the solid fat had a more granular appearance (Van Laack and Smulders, 1989b). In the present experiment we carefully trimmed off all visible fat from the muscles to achieve a low fat content. Fat content of the blend was 12% (assessed through extraction) in both hot and cold processed burgers. We hoped that with such a low fat per

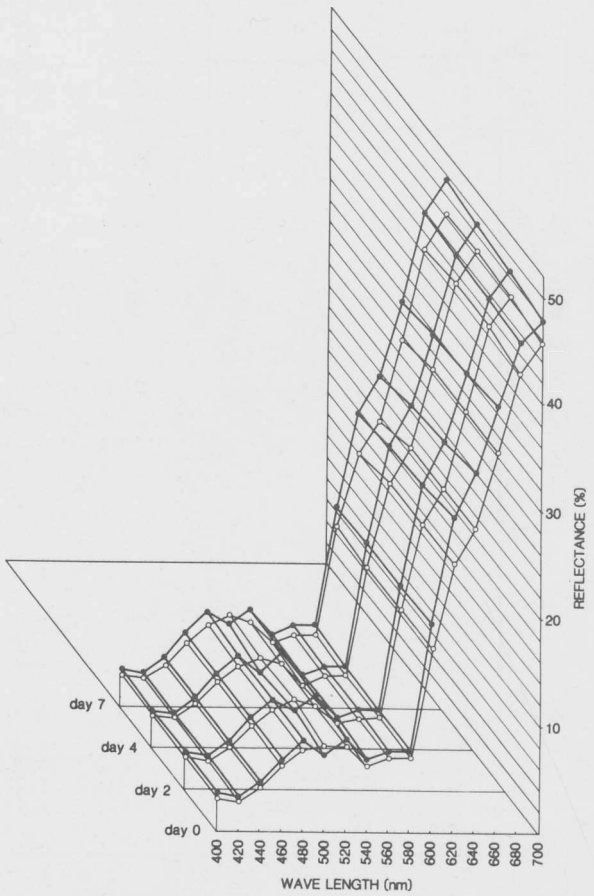


Fig. 2 Reflectance spectra of hot- (•) vs cold- (o) processed hamburgers during 1 week of display at 1±1°C

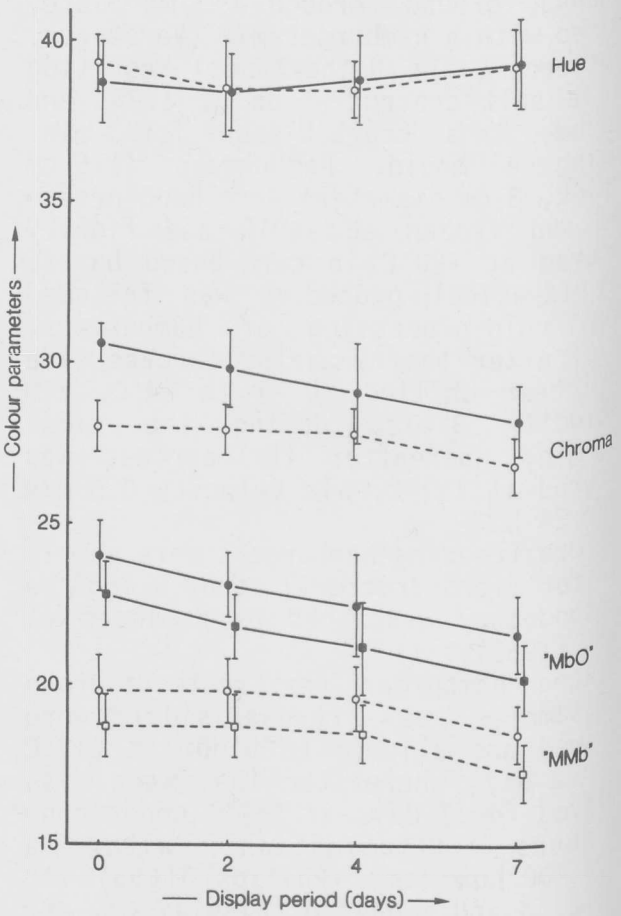
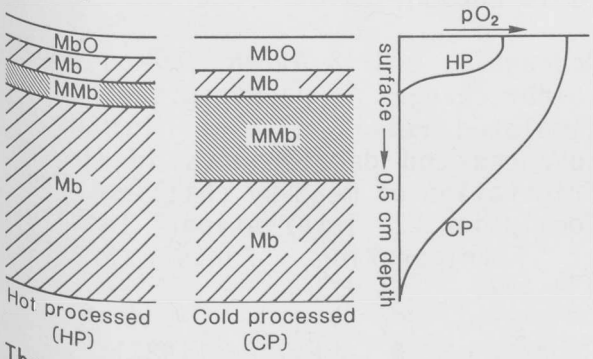


Fig. 3 Hue and chroma values, and % Reflectance (Δ630-580 nm: MbO; and Δ 630-525 nm: 'MMb') of hot- (•) vs cold- (o) processed hamburgers

centage structuraldifferences would interfere less seriously with light reflectance. Yet, light-microscopical examination clearly indicated that the fat in hot, as opposed to cold processed burgers had coalesced in fat channels, finely dispersed through the product. This has likely altered the smoothness of the surface and thus increased the light scatter in hot processed burgers as also observed by Aby-Bakar et al. (1988). Other investigators (e.g. Jacobs and Sebranek, 1980; Seman et al., 1986) found that hot processed ground beef had a darker appearance. One would expect to observe this since hot processed burgers have superior waterholding properties (Van Laack & Smulders, 1989b). It should be realized, however, that, as opposed to the afore-mentioned American studies, the hamburgers in our study were composed of one single ground muscle. It is possible that between-muscle differences account for discrepancies in results since the degree of post mortem protein denaturation differs considerably between muscles, depending on pH and temperature decline and thus on chilling rates. In our study we used sternomandibularis muscle which, on account of its position in the carcass, will chill very fast after hot boning as well as on the cold-to-bone carcass. The MMb reducing activity may therefore have been quite similar for both processing methods (McDougall and Allen, 1986).

Figure 2 is a three-dimensional representation of the light reflectance of hot vs. cold processed beef burgers at different wave lengths, as it changes during one week of refrigerated display. If the observed difference in colour of hot vs. cold processed hamburgers would simply be a matter of altered structure one would expect the reflectance spectra for both types of

products to run more or less parallel. The fact that the curves diverge, converge and even cross at particular wave lengths seems to indicate that the relative proportions and/or location of MbO, Mb and MMb may have been affected by the method of processing. In an earlier study (Van Laack et al., 1987) it was substantiated that the oxygen consumption is higher in pre-rigor than in post-rigor meat. This might lead to a thinner surface layer of MbO. In addition, the lower partial pressure of oxygen in pre-rigor beef, through increasing the redox potential, will increase the reducing capacity in the hot processed hamburgers. This will lead to a thinner layer of MMb and a relatively thick surface layer of Mb (George and Stratmann, 1952). It is tempting to speculate that the relative contribution of Mb to the total reflectance accounts for the higher reflectance values observed in hot processed hamburgers as, strictly for the purpose of illustration of this argument, is visualized below.



The reflectance spectra of muscle pigments are well-documented (Kropf et al., 1976; Hunt, 1980). The total reflectance of meat and meat products being a composite (not simply a sommation!) of the reflectances of the pure pigments, might also explain why only at 500 nm the total reflectance is lower for hot- than for cold processed hamburgers, for it is well possible that at this particular wave length the contribution of, for instance, MMb is less important than that of Mb or MbO.
Particularly at wave lengths >600 nm the reflectances of the hot processed hamburgers decrease more rapidly than those of the cold processed ones (note the difference in convergence of the lines connecting the reflectance values at different storage times on the one hand and the reference lines parallel to the time-axis on the other). This observation indicates that, in contrast with intact muscle (Van Laack et al., 1989; Van Laack and Smulders, 1989a) the colour stability of hot boned, comminuted beef is less stable than that of cold boned comminuted beef. A further substantiation for this is found in the observation that the values for chroma [C*, generally considered a useful parameter to describe colour changes in more descriptive terms (McDougall, 1977)] decrease more rapidly in hot than in cold processed hamburgers (see Figure 3).
In spite of these contrasts it should be recognized, however, that all products, irrespective of way of processing, were very attractive in colour as is reflected by C* values (ca. 27 and higher throughout storage) approximating the reference values generally associated with an attractive, brightly-red, colour (McDougall and Allen, 1986). The fact that display conditions (strict storage at 1±1°C) were almost ideal to preserve colour-stability has undoubtedly contributed to this.

REFERENCES

Abu-Bakar, A., Reagan, J.O., Vaughters, H.M., Campion, D.R. & Miller, M.F. (1988):
Effect of chilling method on the sensory, chemical and cooking characteristics of ground beef patties. J. Food Sci. 53, 1618-1620.

Atkinson, J.L. and Follett, M.J. (1973):
Biochemical studies on the discoloration of fresh meat. J. Food Technol. 8, 51-58.

Brooks, J. (1938):
Color of meat. Food Res. 3, 75-78.

George, P. & Stratmann, C.J. (1952):
The oxidation of myoglobin to metmyoglobin by oxygen. 2. The relation between the first order rate constant and the partial pressure of oxygen. Biochem. J. 51, 418-425.

Hood, D.E. (1980):
Factors affecting the rate of metmyo-

globin accumulation in pre-packaged beef. Meat Sci. 4, 247-265.

Hood, D.E. & Riordan, E.B. (1973):
Discoloration in pre-packaged beef: measurement by reflectance spectrophotometry and shopper discrimination. J. Food Technol. 8, 333-343.

Huffman, D.L. (1980):
Processing effects on fresh and frozen meat color. Proc. 33rd Recipr. Meat Conf., Purdue University, West Lafayette (Ind.) 33, 4-14.

Hunt, M.E. (1980):
Meat color measurements. Proc. 33rd Recipr. Meat Conf., Purdue University, West Lafayette (Ind.) 33, 41-46.

Jacobs, D.K. & Sebranek, J.G. (1980):
Use of pre-rigor beef for frozen ground beef patties. J. Food Sci. 45, 649-651.

Koolmees, P.A., Moerman, P.C. & Zijderveld, M.H.G. (1989):
Image analysis of the fat dispersion in a comminuted meat system. Food Microstr. 8, (in press).

Kropf, D.H., Tuma, H.J., Allen, C.C., Hunt, M.C., Sandberg, M.L. & Schafer, D.E. (1976):
Evaluation of color and other properties of frozen beef. In: Objective methods for food evaluation. Nat'l Acad. Sci. Washington CD, pp. 29-61.

Laack, H.L.J.M. van & Smulders, F.J.M. (1989a):
Colour stability of bovine longissimus and psoas major muscle as affected by electrical stimulation and hot boning. Meat Sci. (subm. for publ.).

Laack, H.L.J.M. van & Smulders, F.J.M. (1989b):
Physical-chemical properties and cooking yield of hamburgers prepared from accelerated processed beef. J. Food Sci. (subm. for publ.).

Laack, H.L.J.M. van, Eikelenboom, G. & Smulders, F.J.M. (1989):
Color stability of hot and cold boned bovine longissimus and psoas major muscle. J. Food Prot. (accepted for publication).

Laack, H.L.J.M. van, Smulders, F.J.M. & Logtestijn, J.G. van (1987):
Air trapping during vacuum packaging of hot and cold boned beef. Proc. 33rd Int. Congr. Meat Sci. Technol., Helsinki, 188-191.

Lawrie, R. (1979):
Meat Science, Pergamon press, Oxford, p. 300-312.

Ledward, D.A. (1985):
Post-slaughter influences on the formation of metmyoglobin in beef muscle. Meat Sci. 15, 149-171.

Ledward, D.A., Smith, G.C., Clarke, H.M. & Nicholson, M. (1977):
Relative role of catalysts and reductants in the formation of metmyoglobin in aerobically stored beef. Meat Sci. 1, 149-156.

McDougall, D.B. (1977):
Colour in meat. In: C.G. Birch, J.G. Brennan, K.J. Parker (Eds). Sensory properties of foods. Applied Science Publ. London, 59-69.

McDougall, D.B. & Allen, D.B. (1986):
Colour changes in packaged beef during simulated retail display. In: Recent advances and developments in the refrigeration of meat by chilling. Proc. Commission C2, International Institute of Refrigeration, Paris (France), 439-447.

Renerre, M. & Labas, R. (1987):
Biochemical factors influencing metmyoglobin formation of beef muscles. Meat Sci. 19, 191-165.

Seideman, S.C., Cross, H.R., Smith, G.C. & Durland, P.R. (1984):
Factors associated with fresh meat color: a review. J. Food Qual. 6, 211-237.

Seman, D.L., Moody, W.G., Fox, J.D. & Gay, N. (1986):
Effects of electrical stimulation and hot boning on color stability of aerobic and vacuum packaged restructured beef steaks. J. Food Sci. 51, 268-272.

Smulders, F.J.M., Laack, H.L.J.M. van & Henrickson, R.L. (1988):
Accelerated processing of beef and

veal. In: B. Krol, P.S. van Roon and J.H.L. Houben (Eds). Trends in Modern Meat Technology 2, Pudoc, Wageningen, 94-102.

Taylor, A.A., Shaw, B.G. & McDougall, D.B. (1980-1981): Hot boning of beef with and without electrical stimulation. Meat Sci. 5, 109-123.

THE INFLUENCE OF DIFFERENT MODERATE COOLING CONDITIONS ON TENDERNESS AND TROPONIN-T-, 30 KDa-, AND TITIN-CONCENTRATIONS OF BULL LONGISSIMUS DORSI.

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INTRODUCTION

We have previously observed a considerable variability in tenderness of young bull longissimus dorsi (LD) under very moderate cooling conditions (Buts et al., 1984, 1986a).
An important part of this variability could be explained by differences in Troponin-T and 30 KDa concentrations determined after separation of the myofibrillar proteins on SDS-Page (Buts et al., 1986b) indicating differences in extent of post-mortal proteolysis as a main cause of tenderness variability.
Maintaining a high temperature early post mortem may result in improved tenderness (e.g. Lochner et al., 1980; Marsh et al, 1981, 1983; Petäjä et al., 1985) probably by enhancement of activity of proteolytic enzymes. On the other hand Tornberg and Larsson (1986) found it is more beneficial to allow meat to go into rigor at 15°C than at 37°C, because the effect of slow rigor development and minimal contraction at 15°C is more important for tenderness of aged beef than the hypothetical effect of increased release of proteolytic enzymes at 37°C.
From this it is clear that temperature during onset of rigor may influence meat tenderness apart from prevention of cold-shortening as it may determine both rate of shortening at rigor and of post mortal proteolysis.
In two experiments temperature during the onset of rigor was varied in different ways in order to study the relation between tenderness and temperature during this important period.

MATERIALS AND METHODS

Animals and treatment of carcasses:
All animals used are from the progeny testing station RSC (B-9258 Scheldewindeke, Belgium). In the first experiment 10 one-year old bulls (mean values ± standard error for live weight: 461 ± 25 kg and dressing-%: 59.4 ± 1.4 %, were slaughtered in the slaughterhouse of our laboratory after captive bold stunning and pithing. Carcass halves were transferred to a cooling room at 4 °C, 0.5 m/sec, one hour p.m. Cooling of right carcass halves was interrupted by moving them to a room at 13°C, 0 m/sec after 3 to 6 h post mortem (p.m.) until 12 h p.m. when they are placed back with the control halves. In the second experiment two groups of animals of 10 and 8 animals respectively, (mean values ± standard error for live weight: 466 ± 45 and 477 ± 34 kg and dressing-%: 57.3 ± 2.1 and 58.8 ± 2.1 % respectively) were slaughtered on different days (one in december and one in april). Control carcass halves (left and right alternating) were cooled at 4°C, 1 m/sec while the other halves were cooled at 13°C, 0.5 m/sec until 8 h p.m. then cooling-room temperature was set to 4°C.
Temperature was measured 7.5 cm deep in the centre of the Longissimus dorsi (8th thoracic rib) either with a portable temperature meter (Technotherm 9503, Instrulab, Brussels, Belgium) or with Pt 100 thermocouples (1/3 Din, Degussa, Brussels, Belgium) connected to a Honeywell recorder outside the cooling room. pH was measured with an Ingold combination electrode (Weilheim, BRD) attached to a digital pH meter (Knick Portamess 651, Knick, Berlin, BRD) at regular intervals post mortem.
In both experiments the LD (8th thoracic rib) was removed ca 24 h p.m. and after trimming of

fat, samples were vacuum packed in polyamide laminated polyethylene (Sidamyl-X, typ EAK 41, Sidac, Ghent, Belgium) and further conditioned at 4°C until 8 days p.m. Then samples were frozen and further preserved at - 18°C for 2 to 6 weeks (exp^{t} 1) or 2 to 3 months (exp^{t} 2). After removal from the deep-freezer cuts were thawed in a standardized way (1 day at 4°C refrigerator and ½ h waterbath at 20°C).

Determinations:
After removal of the cuts from the vacuum bag, drip loss was determined and a subsample of about 50 g covering the whole cutting surface of the sample was removed for isolation of the myofibrillar proteins and subsequent separation on Polyacrylamide gels and determination of sarcomere length (SL). Remaining cuts were then heated in open plastic bags by immersion (1 hour) in a waterbath at 75°C and subsequently cooled under running tap water (ca 10 minutes) and cooking loss determined. Then 20 to 30 cork bore samples (diam. 1.27 cm) were taken parallel to the fibre direction. In exp^{t} 1, 16 samples were used for tenderness evaluation by an eight member trained taste Panel and the remaining samples for the determination of the Warner Bratzler Shear force (WBS) while in exp^{t} 2 all bore samples were used for determination of WBS.

Methods:
Bag drip was measured by the weight difference before and after vacuum packing (sample wiped with a cloth before weighing) and expressed as percentage of initial weight.
Sarcomere length was measured on fresh subsamples of about 10 g fixed in 2.5% glutaraldehyd using laser diffraction as described by Vandendriessche et al. (1984). Average values of measurements of 80 different fibres were obtainded.
Myofibrillar proteins were isolated according to the procedure of Parrish et al. (1973) and isolated myofibrills were dissolved overnight (room temperature, magnetic stirring) in imidazole buffer (0.01 M, pH 7.0) containig 2% SDS and 2% 2-mercaptoethanol. Further preparation of the isolated myofibrils and subsequent electrophoresis was performed as described in Buts et al. (1986b) and Claeys et al. (1989)
After electrophoresis, gels (8 and 4.6 % total acrylamide concentration, 3.1 % crosslinking) were stained with Coomassie Brillant blue and after destaining scanned using a Beckmann model R-112 densitometer. Peak areas were determined and protein concentrations (expressed as BSA equivalents) calculated.
Cooking loss was determined by weight difference before and after heating and expressed as percentage of weigth before cooking.
Warner-Bratzler peak Shear force was determined with a WB-shear mounted on an Instron 1140 Food tester (Instron Ltd., High Wycombe) perpendicular to the fibre direction
Taste panel tenderness rating (exp^{t} 1 only) were obtained using a scale with 8 subdivisions (1 = very tender, ..., 8= very though) as described by Demeyer et al. (1983). The ratings of eight members were averaged.

RESULTS
In table 1 temperature and pH results are shown. For rate of cooling regressions following $Y = a + b\,(1 - e^{-ct})$ (y = muscle temperature °C and t = time post-mortem) were calculated. In this model **a** is the begin temperature at time 0 (theoretical, because the lag time for the installation of the temperature gradient is not taken into acount), **b** the difference between muscle and cooling room temperature at zero time and **c** the rate constant of cooling. The cooling rate at each time post mortem (within the exponential phase of cooling) can be calculated as follows: $dY/dt = bc\,e^{-ct}$ (°C/h). Decimal reduction time (time to accomplish 90% of cooling) can be calcultated as $t_{dec} = \ln(0.1)/-c$, analogous to the prodedure described by Moerman (1973). Decimal reduction time is also expressed on

Table 1: Mean values for temperature, cooling rate characteristics and pH.

	expt 1		expt 2			
			group 1		group 2	
	contr. (n=10)	treatm.	contr. (n=10)	treatm.	contr. (n=8)	treatm.
Temperature:						
1 h	39.0	38.6	32.0	38.9	39.8	39.9
2½ h	33.3	32.9	32.7	33.2	33.6	34.5
4 h	27.1	26.3	25.9	27.6*	27.2	29.5*
6 h	21.3	21.1	19.1	22.6*	21.7	25.4*
8 h	17.2	18.5*	14.6	20.1*	17.4	22.0*
10 h	12.3	15.2*	----	----	----	----
24 h	6.1	6.4	4.9	9.2*	5.5	9.4*
c[1]	0.146	----	0.164	0.145*	0.144	0.117*
t_{dec}[1]	15.8	----	14.1	16.1*	16.3	19.9*
t_{dec}/kg[1]	0.116	----	0.106	0.121*	0.116	0.142*
$t_{10°C}$[2]	14.7	17.4*	11.8	21.1*	13.7	22.0*
pH:						
1 h	6.80	6.82	6.97	6.98	6.79	6.76
2½ h	6.42	6.52	6.48	6.44	6.24	6.05
4 h	6.23	6.16	6.12	6.10	5.87	5.72
6 h	6.12	6.14	5.90	5.85	5.68	5.59*
10 h	5.57	5.68*	----	----	----	----
24 h	----	----	5.60	5.61	5.67	5.64*

level of significance: * at least $P<0.05$ (paired t-test)
[1]: c = rate constant of cooling (1/h); t_{dec}:decimal reduction time (h); t_{dec}/kg: decimal reduction time on carcass halve weight (h/kg).
[2]: time to reach 10 °C (h)

Table 2: Mean values for meat quality characteristics and protein fragmentation

	expt 1		expt 2			
			group 1		group 2	
	contr. (n=10)	treat.	contr. (n=10)	treat.	contr. (n=8)	treat.
Drip-loss (%)[1]	9.9	9.9	6.6	7.5	9.6	9.8
Cook.-loss (%)[1]	26.3	27.4	25.2	25.8	24.7	25.5
SL (µm)	1.87	1.83	1.87	1.89	1.99	1.98
WBS (N)	41.8	44.5	37.7^{a}	33.2^{b}	29.1	30.8
TP score	3.8	3.8	---	---	---	---
Myofibrillar proteins[2]:						
Titin	15.1	15.7	26.6	25.9	27.2	27.4
Troponin-T	7.6	7.1	3.8^{a}	1.4^{b}	1.0	1.5
30 kDa	10.3	11.1	10.9	12.6	15.4	16.1

[1]: For experiment 1, n=7.
[2]: concentration in µg BSA-equival. / mg myofibrillar protein
[a,b]: values with different superscript are significantly different ($p<0.05$; paired t-test) within experiments.
___: significantly different between experiments ($p<0.05$; t-test)

carcass half weight to correct for temperature differences due to differences in muscle weight. The model could not be applied in exp^t 1 because, due to the interruption of forced cooling, no exponential temperature decline was obtained.

From table 1 its clear that in experiment 1 the withdrawal of cooling resulted in higher muscle temperatures for treated halves during a period of time from 8 hour p.m. on, but this temperature difference has disappeared 24 h p.m.

In experiment 2 the difference in cooling conditions resulted in higher muscle temperatures in the treated halves from 4 h p.m. on, a difference that persisted up to 24 h p.m. All temperatures observed in group 1 (slaughtered in december) are lower than the corresponding values in group 2 (slaughtered in april). Furthermore faster rates of post-mortal glycolysis (pH) were observed group 2 as compared to group 1.

From table 2 it is clear that Mean Warner Bratzler Shear force (WBS) was not influenced by the treatment in the first experiment. For the first group of the second experiment mean WBS was lowered and protein fragmentation increased by the treatment. For the second group both control and treated halves were very tender. This was reflected in low Tn-T and high 30 KDa concentrations for both. Titin contents were not different within experiments but very different between expt 1 an experiment 2.

DISCUSSION

Results from exp^t 1 and exp^t 2 (group 1) suggest that a longer period of temperature difference in the early post mortal period is necessary for influence on tenderness, and that the action of temperature on tenderness is related to changes in proteolytic activity. For the second group of experiment 2 it seems that both control and treated halves were cooled slow enough resulting in optimal tenderness for both but there is probably also the effect of higher rate of post mortal glycolysis. The conditions for this group correspond remarkably well with those for which Marsh et al (1987) found the highest tenderness level (pH 3 h p.m ca 6 and corresponding temperature ca 27°C).

Sarcomere length as a measure of shortening of muscles is only very weakly correlated with WBS as is clear from the correlation matrix for all combined data shown in table 3. The significance of the correlation between WBS and SL is due to one (treated) halve of exp^t 1 with SL = 1.53 μ and WBS = 67.4 N, because without this halve the correlation coefficient (r) is -0.215 (not significant). The shorter sarcomere length for this halve clearly did not result from cold-shortening but may be due to so called "heat"- or "rigor"-shortening. The toughness however did clearly not only result from shortening because also the Tn-T and 30 KDa concentrations were relatively high and low (11.2 and 5.5 μg BSA-eq respectively) indicating relatively low post-mortal proteolytic degradation too.

It is clear that conditions for shortening at rigor are about optimal for the control animals. For the treated ones temperatures are somewhat higher (ca 20 °C at pH 5.8 - 6.0 for expt 1 and group 1 of expt 2 and near 30 °C for group 2 of expt 2) than the theoretical optimum of 15-20 °C. It must be mentioned here that temperature is measured in the warmest point of the LD so temperature is lower in the greatest part of the muscle. So it is obvious that, with one exception (see higher), shortening at rigor cannot explain the tenderness differences observed.

On the other hand it is clear from table 3 that an important part of tenderness variability can be explained by differences in proteolytic activity reflected in protein fragmentation. This confirms our earlier results (Buts et al 1986b; 1987) and the results of Seideman & Koohmaraie (1987) who could explain a great part of the variability in tenderness ratings (and shear force values) by differences in fragmentation index.

Table 3: Correlation matrix, mean, standard deviation and range for the poolded date for all halves (n=56).

	Tendern.[1]	WBS	SL	Titin	Tn-T	30KDa
Tendern.[1]	1.000	0.790**	-0.380	-0.245	0.732**	-0.766**
WBS		1.000	-0.338*	-0.030	0.759**	-0.732**
SL			1.000	0.451**	-0.114	0.227
Titin				1.000	-0.113	0.015
Tn-T					1.000	-0.814**
30KDa						1.000
Mean	3.8	36.6	1.90	25.6	3.9	12.5
Stand. Dev.	0.9	9.7	0.14	3.6	4.0	4.3
Min	2.6	20.9	1.53	17.2	0.2	4.4
Max	6.0	67.4	2.26	38.6	14.3	19.3

Level of significance: *: P<0.05; **: P<0.01
[1]: Tendern. = taste panel tenderness; exp[t] 1 only (n=20).

In figures 1 and 2 the relation between WBS and, respectively, Troponin-T and the 30 KDa component is shown. The relations found are not different from the ones we reported earlier (Buts et al, 1986b) for a similar group of animals. The introduction of SL as second independent variable in both relations allowed explanation of a significantly greater part of WBS variability than Tn-T alone (r^2=0.640) but could not explain more than 30 KDa alone.
For the relation between Tn-T and 30 KDa a similar regression equation as before (Buts et al., 1986b, 1987): $Tn\text{-}T_{conc}$ = 13.4 - 0.76 $30KDa_{conc}$ (r^2=0.663, s = 2.36) is found. This highly significant negative correlation may indicate that 30 KDa is a degradation product of Tn-T, but this correlation does not necessarily reflect a direct cause/effect relationship.
The relation between Titin and WBS is unclear and is influenced by the difference in titin concentrations between exp[t] 1 and the two other experiments for which we do not have an explanation. The fact that titin is influenced by a number of factors during storage of samples like time of frozen storage (Greaser et al, 1983) makes it difficult to indicate the contribution of titin in tenderness variability.

CONCLUSION:
An important part of tenderness variability can be explained by differences in (parallel) protein fragmentation (=differences in maturation), although the influence of the structure itself (reflected partly in sarcomere length) cannot be completely excluded even in the absence of cold-shortening.
It is also clear from our results that proteolytic activity can to some extent be influenced by modification of the cooling conditions during installation of rigor. A far better knowledge of the potential hydrolytic activity of proteinases, the activity of proteinase inhibitors and the tenderizing mechanism in general is needed before optimal cooling conditions for maximal tenderization can be indicated.

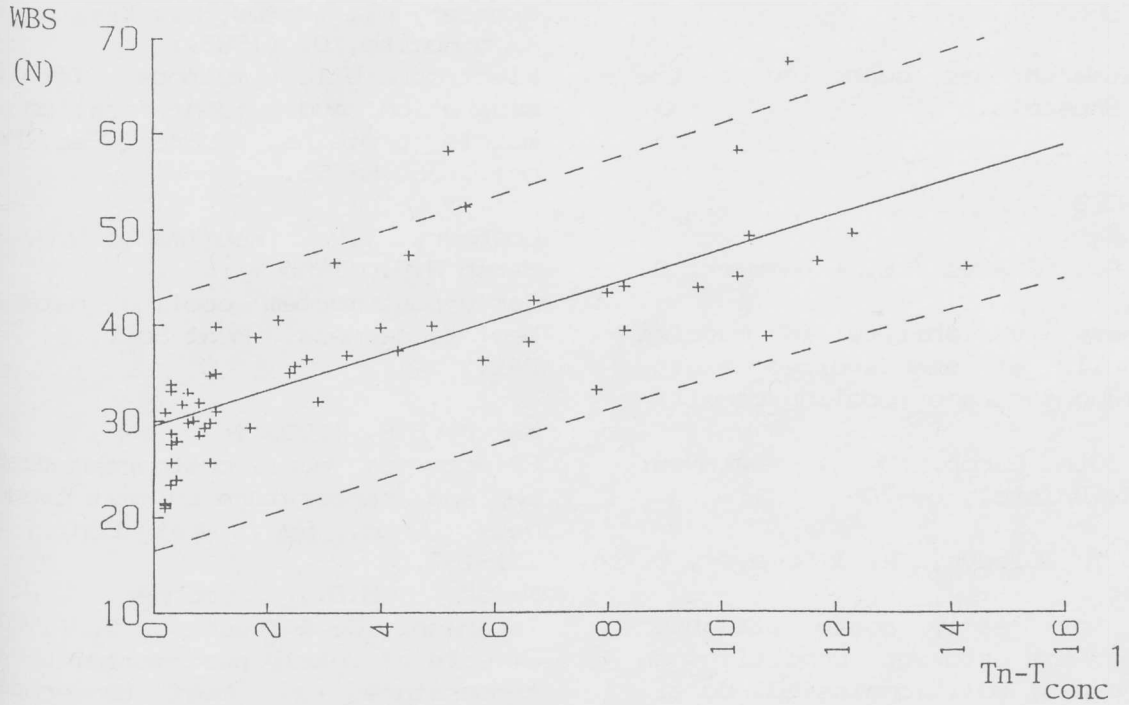

Figure 1 : Regression of WBS on Troponin-T concentr.
Regression equation : WBS = 29.5 + 1.83 $Tn\text{-}T_{conc}$
r^2 = 0.576; S = 6.38 (n = 56)

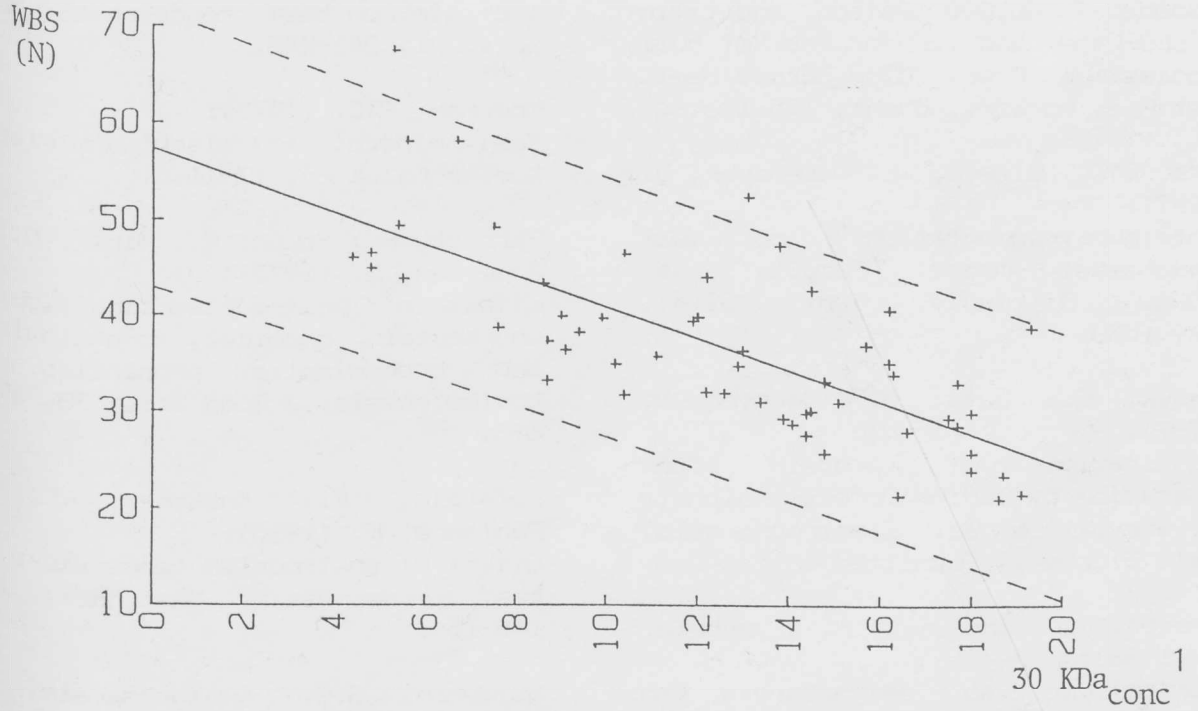

Figure 2 : Regression of WBS on 30 KDa concentr.
Regression equation : WBS = 57.2 - 1.64 $30\ KDa_{conc}$
r^2 = 0.537; S = 6.67 (n = 56)

[1] µg BSA-eq./mg myof. protein

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REFERENCES

Buts, B., Claeys, E. & Demeyer, D. (1984):
Tenderness variability in muscles from bulls at standardized weigth, age, slaughter and cooling conditions.
Proc. 30th Europ. Meet. Meat Res. Workers, Bristol, 69-70

Buts, B., Claeys, E. & Demeyer, D. (1986a):
Effect of early post- slaughter handling and storage conditions on tenderness of bull longissimus dorsi.
Proc. 32nd Europ. Meet. Meat res. Workers, Ghent, 131-133.

Buts, B., Claeys, E. & Demeyer, D. (1986b):
Relation between concentration of troponin-T, 30,000 Dalton and titin on SDS-Page and tenderness of bull Longissimus. Proc. 32nd Europ. Meet. Meat Res. Workers, Ghent, 175-178.

Buts, B., Claeys, E. & Demeyer, D. (1987):
Protein fragmentation and meat tenderness. Meded. Facult. Landbouww. Rijksuniv. Gent, 52(4), 1529-1538.

Claeys, E., Buts, B. & Demeyer, D. (1989):
Quantitation of protein after separation by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis. Anal. Biochem. (Submitted).

Demeyer, D., Hindryckx, P. & Verbeke, R. (1983):
Voorspelling van de malsheid van rundsvlees door meting van een maximale scheurkracht en door sensorische analyse. Landbouwtijdschrift, 36 (2), 279-286.

Greaser, M., Yates, L., Kryzywicki, K. & Roelke, D. (1983):
Electrophoretic methods for the separation and identification of muscle proteins. Proc. Rec. Meat onf., 36, 87-91.

Lochner, J.V., Kauffman, R.G. and Marsh B.B. (1980):
Early-post-mortem cooling rate and beef tenderness. Meat Sci., 4, 227-241.

Marsh, B.B. (1983):
Effects of early-postmortem muscle pH and temperature on meat tenderness. Proc. Rec. Meat Conf., 36, 131-137.

Marsh, B.B., Lochner, J.V., Takahashi, G. & Kragness, D. (1981):
Effects of early post-mortem pH and temperature on beef tenderness. Meat Sci., 5, 479-483.

Marsh, B.B., Ringkob, T.P., Russell, R.L., Swartz, D.R. & Pazgel, L.A. (1987):
Effects of early-postmortem glycolytic rate on beef tenderness. Meat Sci., 21, 241-248.

Moerman, P.C. (1973):
Koelsnelheid van slachtwarm vlees. Koeltechniek 66, 13-16.

Parrish, F., Young, R., Miner, B., & Andersen, L. (1973):
Effect of post-mortem conditioning on certain chemical, morphological and orgqnoleptic properties of bovine muscle. J Food Sci., 38, 690-695.

Petätäja, E., Kukkonen, E. & Puolanne, E. (1985):
Effect of post-mortem temperature on beef tenderness. Meat Sci., 12, 145-154.

Seideman S.C. & Koohmaraie, M. (1987):
Factors associated with tenderness in young beef. Meat Sci., 20, 281-291.

Tornberg, E. & Larsson, G. (1986):
The influence of temperature on the course of rigor and aging in two beef muscles. Proc. 32nd Europ. Meet. Meat res. Workers, Ghent, 85-88.

Vandendriessche, F., Buts, B., Claeys, E., Dendooven, R. & Demeyer, D. (1984):
Sarcomere length measurement by laser diffraction and light microscopy. Proc. 30th Europ. Meet. Meat Res. Workers, Bristol, 110-111.

STUDY OF PURIFICATION AND PROPERTIES OF CREATINE KINASE FROM PORCINE SKELETAL MUSCLES

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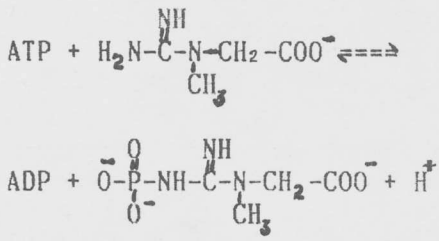
Introduction

Creatine kinase EC 2.7.3.2. is an important enzyme, which participates in the cell energy metabolism and has direct relations with muscle contraction and ATP regeneration. It catalyzes the transphosphorylation reaction between creatine and ATP.



This kinase is found in skeletal muscle, cardiac muscle and brain tissue, presenting three different types, viz.: MM-type (in muscle), BB-type (in brain) and MB-type (in cardiac and other tissues). In recent years, researches reveal that creatine kinase has important role in Na^+-K^+-ATP pump system.(1) Clinically it is also highly evaluated as in the early stage diagnosis of myocardial infarction and in estimating the dimension of infarction.(2) Especially when certain myocardial infarctions can not be judged by electrocardiogram, the analysis of serum creatine kinase is of great use. (2),(3). Therefore, the study of the purification and properties of creatine kinase has significance not only in theory and also in practice.

In 1954 Kuby et al firstly purified and crystallized this enzyme from rabbit liver. (4).Thereafter, a series of isolation and purification works were performed from muscles and brains of various animals e.g. rat(5), monkey(6), carp(7), chicken(8) and human being(9) and horse (10). Among those, the study of creatine kinase from rabbit muscle was the most detailed. The physico-chemical properties, the catalytic mechanisms and the structure of subunits were all studied. (11), (12), (13). Recently, along with the development of molecular biology, even the amino acid sequence of creatine kinase from rabbit, chicken and rat were analysed. (14)(15). But studies of creatine kinase from pig are more less. For pig has a great bulk of muscles, and pig muscle is a good source of many biomolecules, moreover pig is nearer to human beings in biological species, so it is appropriate to isolate and purify creatine kinase from pig muscles instead of from muscles of other minor animals.

Materials and methods

Materials:
Fresh muscles from Hangzhou Meat Factory
Chemical reagents: All A.R. grade or biochemical grade
Apparatus: Centrifuges, visible & ultraviolet spectrophotometers, partition collectors, AA-analyzer (Hitachi 835-50)

Methods:
1) Isolation and purification procedures
a) Fraction I Crude extract
Minced pig muscles (back and leg) 400g. are homogenized with 0.01M.KCL solution (2ml./g.muscle) and then centrifuged (3000 r.p.m.). The supernatant is filtered through 4 layers of cheesecloth. The solution is the crude extract.
b) Fraction II 60% ethyl alcohol-$MgSO_4$ precipitation
The pH of the crude extract is adjusted with 5M.ammonia solution to 9.0. Solid NH_4Cl is added till the concentration is brought to 0.1 M., and adjust pH to 9.0 once more. After stirring for 1hr., 95% ethyl alcohol is added at -10 ℃., to a concentration of 60% (taking the 95% alcohol as 100%). Stirring is continued for half an hour. The solution is warmed to 10℃., centrifuge (3000 r.p.m.)for 15 minutes. To the supernatant, pH 8.5, 2.0 M $MgSO_4$ solution is added to the concentration of 0.03 M.. Restore the concentration of alcohol to 60%, stir for 15 minutes at about 10℃.. Centrifuge for 30 minutes (4000 r.p.m.). The precipitate is extracted twice with pH 9.0, 0.07 M. $Mg(Ac)_2$(The amount for the first time is 8% of that of crude extract, for the

second time is 4%). Centrifuge for 30 minutes (4000 r.p.m.). Combine the supernatants and add 95% ethyl alcihol at -10℃. to the concentration of 50%. Stir for 30 minutes, centrifuge at -5℃.for 30 minutes (4000 r.p.m.). The precipitate is dissolved in pH 8.0, 0.01 M. tris buffer solution.

c) Fraction III $(NH4)_2SO_4$ precipitation (0.45-0.75 saturation)

The above solution is dialysed against the same tris buffer solution for 24 hrs. Centrifuge for 30 minutes (4000 r.p.m.). To the supernatant, $(NH4)_2SO_4$ is added to 0.45 saturation, while 1 M. NH4OH is added to maintain the pH to 7.8. Put aside for 2 hrs, centrifuge for 30 minutes (4000 r.p.m.) To the supernatant $(NH4)_2SO_4$ is again added to 0.75 saturation. Stand by for 3 hrs. Centrifuge for 30 minutes (11000xg.). Dissolve the ppt.in the same buffer solution and dialyse to remove $(NH4)_2SO_4$. And then use pH 8, 0.02 M. tris solution containing 0.01 M. mercaptan to equilibrate.

d) Fraction IV DEAE chromatography

Use 0.02 M. pH 8.5 tris buffer to equilibrate the DEAE column (20x1.5 cm.). Add sample in 3 batches. At room temperature (8℃.), use 0.02 M. tris and 0.1 M. tris buffer (pH 8.1) 100 ml. each to elu ate. Collect in tubes, 7 min. one tube (4 ml.). Combine the eluates which show enzyme activity. Add $(NH4)_2SO_4$ to 0.9 saturation, centrifuge, suspend the ppt. in small amount of $(NH4)_2SO_4$ soln. with the same degree of saturation. Keep at -25℃.

2) Determination of enzyme activity

a) Creatine color reaction method (16)

b) pH method (17)(18)

3) Protein determination Biuret method

4) PAGE method for purity determination (19)

5) Sephadex gel chromatography for molecular weight determination (19)

6) SDS-PAGE method for molecular weight determination (19)

7) Amino acid analysis (20)

8) Tryptophane determination (19)

RESULTS

1) Purification and identification of creatine kinase

From 400 g. of pig muscles, 30 ml. of creatine kinase solution (16 mg.protein /ml.) were obtained. Activity recovery was 42.5%, specific activity was 85 units/1mg. protein. (table I)

step / Item	I crude extract	II Alc. $MgSO_4$ ppt.	III $(NH_4)_2SO_4$ ppt.	IV DEAE chromat.
Vol. (ml.)	600	87	68	30
total protein	11080	1183	734	480
total activity	96000	55680	47056	40800
recovery %	100	58.6	49.0	42.5
specific activity units/mg. protein	8.66	47.06	64.10	85.00
purification fold	1.0	5.42	7.40	9.82

Table I Purification of creatine kinase

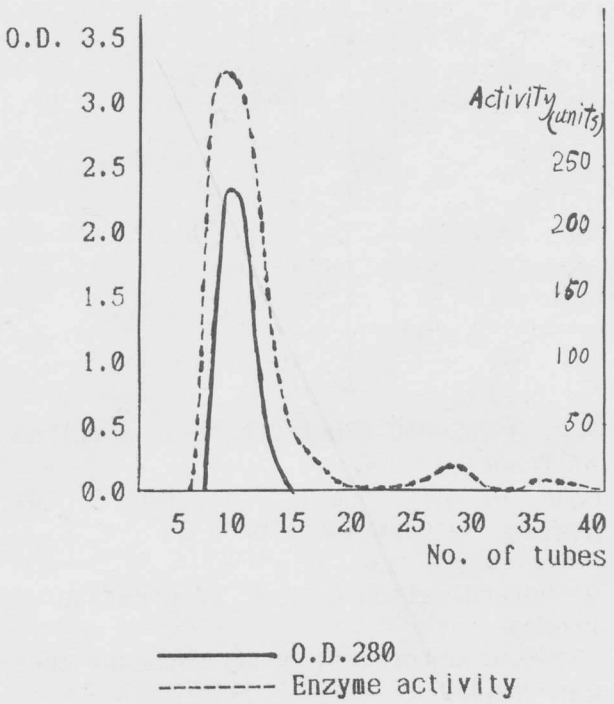


Fig.1 DEAE chromatography

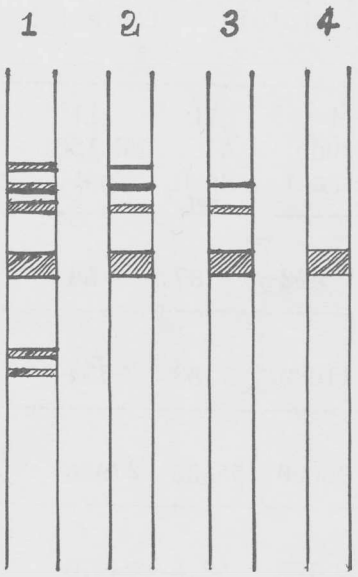


Fig.2 PAG-electrophoretic spectrum
Conc. of gel 7%,
stained by aniline black
Tube 1,2,3,4 correspond the fraction I, II, III, IV respectively
Tube 4 (one band only) shows the enzyme preparation has attained electrophoretic pure

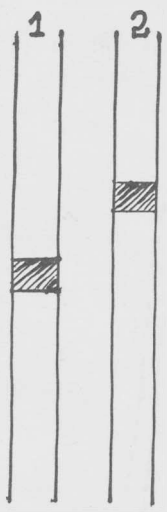


Fig. 3 SDS-PAG electrophoretic spectrum of fraction IV
Conc. of gel: Tube 1, 7.5%, Tube 2, 10%, stained by commaise blue R-25

2) Determination of M.W. of creatine kinase

a) M.W. determination by sephadex chromatography

The M.W. of creatine kinase 83,000 was obtained from standard curve made from the data on table II.

Item / Proteins	M.W.	Vol. of eluate
horse liver dehydrogenase	80,000	27
bovine serum albumin	67,000	32
horse radish peroxidase	40,000	43
trypsin	23,300	52.5
sample	83,000	25

Table II Volume of eluate of standard proteins and sample protein

b) M.W. by SDS-PAGE-method

The M.W. of creatine kinase subunit, 42,000 was obtained from the standard curve made from the data on table III.

The relative motility of sample was 0.525, the corresponding M.W. was about 42,000.

Item	dye frontier	band dist.	relative motility	log M.W.	M.W. x10
phosphorylase B	6.4	1.2	0.187	4.937	9.40
TMV	6.4	5.8	0.906	4.243	1.75
carbonic anhydrase	6.4	4.5	0.703	4.447	3.00
actin	6.4	3.2	0.500	4.633	4.30
bovine serum albumin	6.4	2.2	0.343	4.826	6.70
sample	6.1	3.2	0.525	4.630	4.20

Table III Band distances (cm.) of standards and sample

3) Amino acid analysis

a) Tryptophane content

50mg./g.protein obtained from the standard curve.

b) Total amino acids analysis (except Trp.) by amino acid analyzer

amino acid	content mg.% protein	amino acid	content mg.% protein
Asp.	113.33	Cys.	9.95
Thr.	37.875	Val.	56.34
Ser.	37.837	Met.	28.71
Glu.	118.47	Ile.	26.53
Ala.	26.37	Leu.	86.74
Gly.	46.76	Tyr.	13.22
Phe.	47.43	Lys.	89.18
His.	29.78	Arg.	47.40
Pro.	33.07		

Table IV Amino acid Analysis results

4) Kinetic properties of pig muscle creatine kinase

a) Optimum pH

The optimum pH of this enzyme is between 6.5~7.0, obtained from the figure 4 based upon the data on table V. The enzyme activity was determined by creatine color reaction method. Enzyme conc.0.06 mg. protein /ml..

Item pH	Activity	Activity ratio
5.5	2.975	35
6.0	5.950	70
6.5	7.990	94
7.0	8.500	100
7.5	5.100	60
8.0	2.215	25

Table V The influence of pH on creatine kinase activity

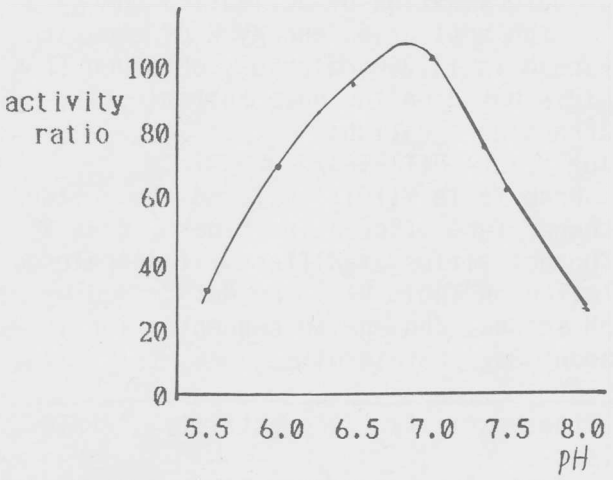


Fig.4 The optimum pH of creatine kinase

b) The influence of temperature on creatine kinase

Keep creatine kinase solution at different temperatures for 15 minutes, the inactivation is significant at 60 ℃. (Table VI, Fig. 5)

activity	before incubation	after incubation	ratio
20° C	60	60	100%
25° C	60	60	100%
30° C	60	60	100%
35° C	60	60	100%
40° C	60	53	88%
45° C	60	50	83%
50° C	60	30	50%
60° C	60	0	0

Table VI The influence of temperature on creatine kinase activity

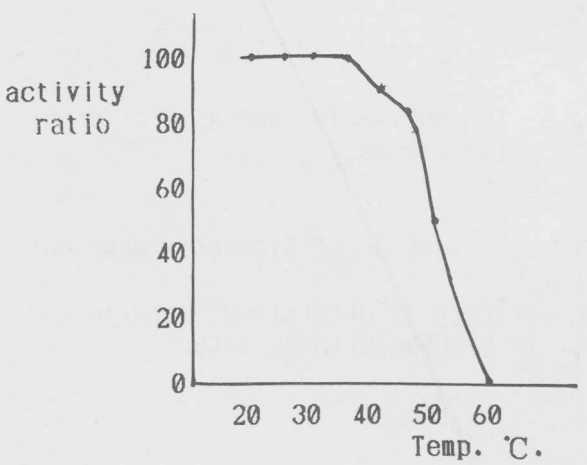


Fig.5 The influence of temperature on creatine kinase

c) Determination of activation energy E

The activation energy E of creatine kinase is 14,280 calories, obtained from Fig.6 based on the data on table VII, by Arrhenius eguation:

$\log a_2/a_1 = E(T_2 - T_1)/2.303\ RT_1T_2$

From Table VII it is found the optimum temperature of creatine kinase is 35 ℃. The activities at different temperatures (cited on Table VII) are determined by pH-method. The enzyme concentration is about 3mg.protein/ml.

Item Temp.	activity	log activity	1/Tx10
15 ℃	48	1.681	34.7
20 ℃	60	1.778	34.2
25 ℃	110	2.041	33.5
30 ℃	170	2.230	33.0
35 ℃	240	2.380	32.5
40 ℃	200	2.300	31.9

Table VII The activity under different temperatures

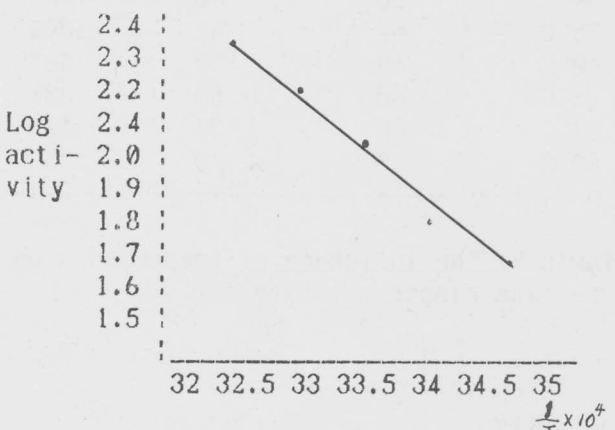


Fig.6 The activation energy E of creatine kinase

d) Determination of Michaelis constant Km

The activity is determined by pH-method, plot as Lineweaver-Burk method.

(1) Km-ATP

Creatine is fixed at 40 mM. 1/Km is obtained from Fig.7 and hence Km=2.04 mM.

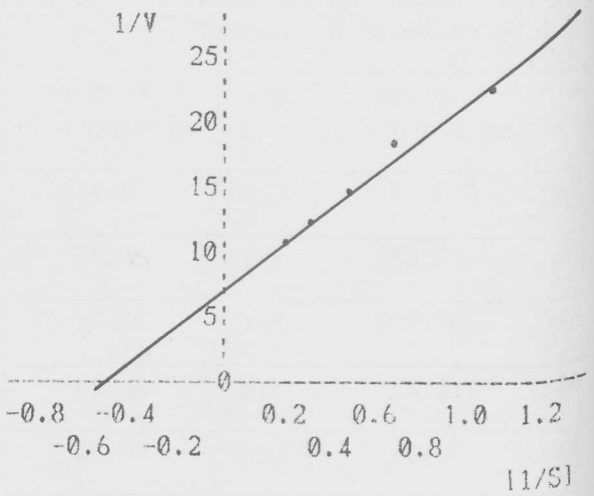


Fig.7 Lineweaver-Burk plot of Km-ATP

(2) Km-creatine

ATP concentration is fixed at 4mM/3ml. 1/km is obtained from Fig.8, hence Km-creatine equals 14.28mM.

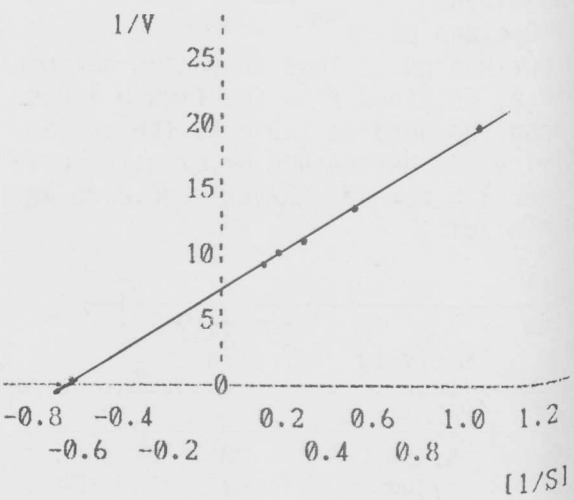


Fig. 8 Lineweaver-Burk plot of Km-creatine

DISCUSSION

In 1954, Kuby et al (4) firstly isolated and purified creatine kinase from rabbit muscle. Owing to the crystallization process was more time-comsuming, many modifications were performed. In 1981 Toshihide and Takasawa (21) purified without crystallization, creatine kinase from pig muscles with DEAE ion exchange chromatography and CM-cellulose chromatography. The activity recovery was 41%, purification fold was 8. We used fractional precipitation method with alcohol & ammonium salts, and DEAE ion exchange chromatography method, in condition that no cold room was available, got a satisfactory result--activity recovery 42.5%, purification fold 10. The M.W. of porcine creatine kinase 83,000, we obtained by sephadex chromatography, is nearer to that of rabbit creatine kinase,82,000. (23) The M.W. of subunit by SDS-PAGE method, is 42,000. The reproducibility of these two methods was high. The amino acid analysis results of porcine creatine kinase were nearer to that of rabbit creatine kinase. (24) The optimum pH, heat stability were nearer to those of chicken (25) and human (26) creatine kinase. Activation energy, Michaelis constant are all nearer to those of rabbit creatine kinase. (21) Therefore it is highly feasible to use porcine creatine kinase instead of rabbit creatine kinase in scientific and clinical practice.

REFERENCES

1) Saks,V.A.et al (1977) Biophs. Acta. 463 (3), 550
2) Inst. of Cardio-vascular Diseases, Chinese Medical Acad. (1974) J. of Card. Vasc. Dis. 2(4), 278
3) Sobel, B.E. et al (1972), Circulation, 46, 640
4) Kuby, S.A., Noda,L. & Lordy, H.A. (1954), J.Biol.Chem. 209, 191-201
5) Hooton, B.T. & Watts, D.C. (1966) Biochem. J. 100, 637-646
6) Kumudavalli, I., Moroland, B.H. & Watts, D.C. (1970) Biochem. J. 117,513-525
7) Gosselin-Rey, Cetal (1970) Biochim. Biophys. Acta 221, 241-254
8) Roy, B.P., Laws, J.F. & Thomson, A.R. (1970)
9) Keutel, H.J., Okabe, K., Jacobs, H.K., et al (1972) Arch. Biochem. Biophys. 150, 648-678
10) Takasawa, T., Fukushi, K. et al (1981) J. Biochem. 89, 1619-1631
11) Watts, D.C. The Enzymes, 1962,8,384
12) Kuby, S.A. The Enzymes, 1962,6,515
13) Bicherstaff, G.F. et al J. Biochem. 1978, 91
14) Pamela, A.B., Robert, A.Z. et al (1984) J.Biol. chem. 259 (23), 14979-14984
15) Arther E.J. and Pavid, P. (1984) J. Biol.Chem. 259, (24), 14985-14992
16) Clinical Biochemical Tests and Determinations Part 1, P.338, 1984, Edited by Shanghai Municipal Medical Labortory
17) Zeng Shi-yuan et al (1986) J.of Hangzhou Institute of Commerce, Natural Science Edition, Vol.I, P.28-30
18) Yao Qi-zhi and Zou Chen-lu (1981) An assay method for creatine phospho kinase activity (pH colorimetry) J.of Biochemistry and Biophysics (China) 13,1
19) Zhang Long-xiang et al Biochemical Methods and Technology, Higher Education Edition (1985) P.94, 112, 124
20) Hua Jia-sheng et al Practical Techniques of Protein Chemistry, Science Edition (1980)
21) Takasawa, T. and Fukushi, K. (1981) J.Biochem. 90, 195-204
23) Yue, R.H., Palmieri, R.H., Olson, O.E. & Kuby, S.A. (1967) Biochemistry, 6, 3204-3227
24) Ouchterlony, C. (1949) Acta. Pathol. Microbiol. Scand. 26, 507
25) Hans, M. Eppenberger et al (1967) J.Biol.Chem. 242,(2),204-209
26) Cheng Lan-ying et al (1981) J. of Biochemistry and Biophysics (China) Vol.I, 17-27